APPLICATION OF THE ADHESIVE TAPE METHOD FOR MICROBIAL SAMPLING ON VARIOUS MEAT SURFACES

by

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INTRODUCTION

Food industry concerned with food plant sanitation, food spoilage, and food preservation need to monitor the microbial load (level and type) on surfaces of food products and in food processing environments. From this information, they are able to evaluate the quality of food products, efficiency of cleaning and sanitizing compounds, and assess the overall sanitary condition of food processing areas. Carcass meat is a solid structure with microbial growth generally confined to the outer surfaces (Brown, 1982). Many of the methods developed have been evaluated on the basis of their ability to produce the highest counts possible. However, for practical purposes it seems more important to obtain a figure which gives a reliable indication of the average contamination of the whole surface in question, rather than the highest possible recovery from some samples (Hansen, 1962).

Conventional ways of making a viable cell count from surface are laborious and time consuming. Swab, rinse, and excised-blending are most widely used techniques for examining the surfaces of poultry and red meats. They all require dilution, plating, and 24 to 48 hr incubation procedures.

A simple and rapid adhesive tape method was developed to provide reliable estimation of the level of microbial contamination on meat surfaces. The purposes of this study were to simplify meat surface sampling procedures to obtain a reliable estimation of contamination and test the feasibility of the tape for different types of meat surfaces; secondly, to study the correlation between adhesive tape method and two conventional methods (swab method and excised-

blending method); and thirdly, to evaluate off-odor of meat in relation to microbial level on meat surfaces and to predict the shelf life of raw meats.

LITERATURE REVIEW

Surface Sampling for Microbial Contamination

Surface samples provide a useful index of the extent of microbial contamination of the meat, because the surface is the area most likely to be cross-contaminated during handling or evisceration and to be affected by high humidity or any short-term temperature fluctuations during storage, and also because the interior of muscle is usually regarded as being sterile (Brown and Baird-Parker, 1982). The surface microbial count of stored meat samples increases as a function of time and storage temperature. Useful information concerning the keeping qualities at a given temperature can be obtained by surface counts and in certain cases by visual inspection. Microbiologists have been concerned with the detection and enumeration of microorganisms on surfaces for over 60 years. Numerous procedures have been described for the determination of the microbiological condition of meat surfaces and meat products. Vanderzant et al. (1976) stated that several factors should be considered in the selection of a bacteriological testing procedure: type of sample, objective of the test, microbial levels expected, presence of bactericidal compounds on surface of sample, resources available for sampling and laboratory analysis, environmental conditions during sampling and precision and accuracy required, with objectives of the test being especially important.

Meat sampling methods usually can be classified as non-destructive and destructive sampling.

I. Non-Destructive Microbial Sampling Methods

1. Swab method

In 1917, Manheimer and Ybanez developed a swab-rinse technique for assessing bacterial contamination on eating utensils (Favero et al., 1968). It employs a template to delineate the area to be swabbed. Thorough rubbing with wet and/or dry swabs provides the abrasion to dislodge microbes from the surface.

The swab method has been modified in many ways. Calcium alginate swabs to replace the cotton swab were tested by several investigators. Calcium alginate dissolves in Ringer's solution and sodium hexametaphosphate, and thus frees any microorganism entrapped and produces higher counts than the cotton swab (Fromm, 1959; Higgins, 1950). Fromm (1959) evaluated a number of techniques, and concluded that the alginate swab was the method of choice considering accuracy, variability, ease, and speed of manipulation. However, certain researchers contended that cotton is superior to alginate since alginate or sodium hexametaphosphate may be inhibitory to some microorganisms (Emswiler et al., 1978; Favero et al., 1968). A membrane filter was applied together with swab method by many investigators. This involves filtering the calcium alginate swab sample solution through a membrane filter which is then placed on a growth medium, incubated, stained, and observed under microscope. Angelotti et al. (1958) reported that higher recovery was obtained when an analyst swabbed several similar sites with the same swab rather than swabbing a single site with a single swab. This will help average variations of counts between similar areas with non-uniform distribution of bacterial cells. Greene et al. (1962) described a modified swab method called "swab-pression". The device consists of a replicating floc material, such as velveteen, mounted on a curved section of metal. The floc is cut to a definite size and mounted by double stick

tape. The sterilized device is rolled over nutrient agar surface to moisten the floc and next rolled once over the test surface and finally rolled over a nutrient culture agar that is subsequently incubated.

The disadvantages of the swab method are incomplete and inconsistent removal of surface bacteria for enumeration. Incomplete removal may be the result of incomplete release of bacteria from the swab during shaking of the diluent. Inconsistency may be caused by many variables, such as pressure applied to the swab, moisture content of the swab, the length of swabbing time, fat content and texture of the surface (Avens and Miller, 1970b; Baldock, 1974; Fromm, 1959; Ulrich, 1964). Usually surface counts are done when accuracy is not of the utmost importance; the convenience and absence of carcass damage caused by swabbing must be balanced against the accuracy required.

2. Rinse method

With this method the contaminated surface is either immersed in a sterile fluid, or the fluid brought into contact with the surface being examined. The method is more precise and accurate than the swab method because the entire surface is tested (Angelotti et al., 1958; Baldock, 1974). Sampling an entire area reduces error and yields higher recoveries by eliminating error introduced by inconsistent or non-uniform contamination. Recovery from the surface may be low if the surface tends to retain the bacteria. Thus with poultry skin, recovery by this method is low unless the sample is shaken in a bag with an abrasive material such as rough sand in the sterile diluent.

There were also some modifications of rinse methods. Clark (1965a, 1965b) prescribed a spray gun technique in which a spray of sterile rinse fluid was impinged against a circumscribed area, thus removing the microbial contaminants. The rinse fluid was recirculated to a reservoir, subsequently plated and total

bacteria count related to unit surface area. This method was reported to yield higher total bacteria counts than the swab or skin blending method when used on freshly inoculated food surfaces. Stainless steel strips have been employed to assess the level of air-borne microorganisms which accumulate on surfaces (Favero et al., 1968; Whitfield et al., 1969). With this method, sterile stainless steel strips were placed in the same environment as the surface; after a determined period of environmental exposure, the strips were then transferred to bottles containing 1% sterile peptone water and subjected to ultrasonic energy or mechanical agitation. The rinse fluid was then plated with an agar medium. The drawbacks of this method are that it is an indirect method and that it may be inaccurate when the level of microbial contamination is low.

Angelotti et al. (1958) concluded that rinsing with concomitant scrubbing is better than APHA cotton swab (1 swab per 4 surfaces), cotton swab (1 swab per single surface), alginate swab, agar syringe method and direct surface agar plate method. The disadvantages of the rinse method are the lack of friction to remove microorganisms from the skin and prevention of water to dislodge bacteria by skin fat (Fromm, 1959). It is difficult to use total carcass counts for comparison due to lack of uniformity of carcass sizes and inconvenience of making counts of such large objects.

3. Direct agar contact

In this method solidified nutrient agar surface is pressed against a sample surface to be examined, removed, covered, and incubated before counting colonies on agar. The Rodac® agar plate system (Div. Becton, Dickinson & Co., Oxnard, CA) is the most widely used agar contact method due to its commercial availability, simplicity and potability (Baldock, 1974; Favero et al., 1968; Hall and Hartnett, 1964). Litsky (cited by Walter, 1955) developed a syringe-like

apparatus with a large barrel filled with sterile agar. A plunger pushes the column of agar out so that the agar contacts the test surface. After contact, the outer layer of agar is cut off and placed in a petri dish for incubation. The major drawback of this technique is the difficulty in obtaining a flat smooth surface on the agar after cutting. Instead of a syringe, artificial sausage casing can also serve as a container for agar. Agar contact methods provide a pictorial demonstration of the presence of bacteria on a variety of surfaces (Baldock, 1974). Greene and Herman (1961) filled aluminum milk bottle covers with agar, and directly applied these agar "plates" to test surfaces. Compared with the agar syringe method of Litsky (cited by Walter, 1955), these "plates" provided consistent flat agar surfaces for sampling and are inexpensive and in plentiful supply. A reflectance method using Rodac® plates was developed by first measuring the reflectance of pre-poured agar. After obtaining samples from surfaces and incubated contact plates at 30°C for 48 hr, the reflectance of each plate was again measured. By design, bacterial colonies when present on the plate turn red and lower the reflectance (whiteness) of the medium. By use of standard curves, bacterial counts per square inch can be estimated by the difference in reflectance. The primary advantage of the method is the ease of estimating high levels of bacteria without the need of dilution, because is impossible to count colonies on the standard Rodac® contact plate when bacterial contamination is very high (Jedlicka and Hill, 1975).

Angelotti et al. (1958) indicated for flat or slightly rounded nonporous surfaces with fairly uniform contamination, agar syringe method appears to be better than swab method. However, cotton swab method is more versatile on surfaces to be applied. The data also showed that although agar syringe method provides low recovery of bacteria, it has excellent precision. Niskanen and Pohja (1977) compared recovery of bacteria from stainless steel, plastic, wood, and

meat surface by the contact plate and swab methods, and found that for a flat, firm surface the contact plate method provided better recovery and repeatability. The swab method was more appropriate for flexible and uneven surfaces and for heavily contaminated surfaces. The disadvantages of direct contact method include limitation to surfaces of low contamination and the interference of counts by confluence growth of mold or spreading colonies (Goulet et al., 1983).

4. Direct surface agar plate method

Contaminants on surfaces can be detected on site by the direct surface agar plate (DSAP) method, where sterile melted agar is poured on to the tested surface and left to solidify under a sterile cover. After incubation the colonies at the agar interface are counted. Small items can be placed in a petri dish and covered with agar (Angelotti and Foter, 1958; Baldock, 1974; Patterson, 1971). This is mainly a laboratory technique since food plant surfaces are generally large, fixed, and cannot be incubated at a desired temperature. Moreover, it cannot be used on surfaces containing residual amounts of bactericidal or bacteriostatic chemicals that would inhibit the growth of microorganisms. Also, the method is not reliable on heavily contaminated surfaces because resultant colonies will coalesce (Baldock, 1974; Favero et al., 1968; Patterson, 1971). Angelotti and Foter (1958) compared the precision of the DSAP method with that of a conventional plate count technique for detecting the bacterial contamination on nonporous surface. They showed that the variation between replicate counts of both methods was equivalent with either spores or vegetative cells. DSAP had the advantage over other recovery methods because in situ determinations are made, thus eliminating errors associated with intermediate manipulations.

5. Scraping method

Scraping is also a useful sampling method and has been suggested for poultry, cattle, and sheep carcasses and meat cuts (Patterson, 1971). Williams (1967) has suggested a scraping method involving pipetting diluent into a hollow cylinder which was held with its open end tightly against the surface. The meat surface below the diluent was scraped with a sterile spatula to release maximum suspension of bacteria in the solution. The results showed that higher surface viable counts on meat were determined by this method than the cotton swab method by which the latter removed generally <10% of the organisms present. Goulet et al. (1983) have tested a new method for sampling meat surfaces using abrasive discs against the spray gun technique described by Clark (1965a, 1965b). A special stainless steel holder enabled the user to remove one disc from the sterile tube and held it against the horizontal or vertical surface to be sampled. After sampling, the disc was released into sterile peptone solution and where shaking, dilution, and plating were made. This method was slightly less efficient than the spray gun in recovering bacteria during the early stages of incubation but was about 600% more efficient after 72 and 120 hr. Goulet et al. (1983) explained that when the bacterial population began to multiply and the metabolic activity increased, the cells became more incrusted into the meat through enzymatic action. It was during this second phase of bacterial activity that the abrasive discs showed a definite advantage over the spray gun. They also believed that this tool was a significant improvement over most of the surface sampling techniques already used for meat and meat products.

6. Vacuum method

The vacuum method has been used experimentally to evaluate microbiological contamination in hospital carpeting (Favero et al., 1968). It employed a canula connected by rubber tubing to either a slit sampler or an all-glass impinger. Under vacuum, particles containing microorganisms were removed from the carpeting and impinged against an agar surface in the slit sampler or into a liquid medium in the impinger. Whitfield et al. (1969) reported that the need for a device to sample large surface areas that were lightly contaminated with microorganisms motivated the development of the vacuum probe sampler which utilized airflow through a critically sized orifice to remove particles from surfaces and a membrane filter to capture these particles. The results showed that particle removal efficiencies were consistently in excess of 80%, with a mean removal efficiency of 89%.

7. Radiometry

Bacteria tagged with radioisotopes have been used in laboratory studies to determine factors necessary for obtaining microbiologically clean surfaces. This technique has proved to be both sensitive and accurate with most surfaces. Ridenour (1952) was able to demonstrate that different organisms adhere to the same surface with different tenacities and are not removed with the same ease. Furthermore, it was shown that the same organisms would adhere to different surfaces with different tenacities.

II. Destructive Microbial Sampling Methods

Destructive sampling methods involving sample removal and maceration (blending or rinsing) are by far the most efficient means of removing microorganisms from meat for counting (Emswiler et al., 1978; Mead and Thomas, 1973). Fromm (1959)

quantitatively compared the four basic sampling methods on chicken carcasses, and the results indicated that the skin tissue removal method was the most accurate. The skin tissue removal method reported by Avens and Miller (1970b) involved removing a known area of skin or meat surface from the carcass with a sterile cutting tool and agitating or comminuting with an appropriate dilution fluid in a sterile laboratory blender. The fluid was then diluted and plated for subsequent colony counts.

The optimum skin blending for quantifying poultry carcass skin bacteria was determined by Avens and Miller (1970a). They found that turkey carcass skin samples blended in peptone water or physiological saline solution yielded significantly higher bacteria counts than skin samples blended in Butterfield's buffered-phosphate diluent or deionized water, regardless of blending time and it made no significant difference whether samples were blended for 1, 2, 3 or 4 min. They concluded that peptone water (0.1% peptone) is the optimum blending diluent fluid and 2 min is an optimum blending time for use in quantifying bacteria on poultry carcass skin. A comparison of massaging meat with an instrument called Stomacher® (stomaching) and conventional blending techniques for the homogenization of excised meat samples for microbiological analysis was reported by Sharpe and Jackson (1972) and Emswiler et al. (1977). Bacterial counts did not differ significantly between the Stomacher® and blender for each type of meat studied. The use of a Stomacher® in the microbiological examination of meat products is favored because labor involved in cleaning and sterilizing reusable jars and blades is reduced, reduction of aerosol formation, minimal temperature rise and damage to microbial cells, and the small storage space required for stomacher bags (Emswiler et al., 1977).

Berry et al. (1978) used non-destructive swabbing and destructive tissue removal coupled with fluid agitation (shaking) techniques to evaluate bacterial

recovery from beef, pork, and lamb adipose tissue. From their investigation, higher counts were obtained with a tissue removal and shaking method than with surface swabbing, but only when bacterial levels were low. They suggested that bacterial recovery by both methods was unaffected by species and differences in surface texture, sample storage time, and duration of fluid agitation. Swabbing may be preferable to shaking as routine procedure for large numbers of samples because it reduces sampling time. However, Lazarus et al. (1977) who compared microbial counts on beef carcasses by using the moist swab contact and secondary tissue removal methods, found that when the microbial count was higher than log 4.5/6.45 cm^2, the tissue removal rinse technique provided a better indication of the true microbial population for given surface area than the moist-swab method. But when microbial numbers were less than log 4/6.45 cm^2, comparable results were obtained regardless of the method employed. Notermans and Kampelmacher (1975b) studied sampling methods used to monitor hygiene at every stage in poultry processing and concluded that the maceration method (excised-blending) gave a better estimate of the hygiene conditions than the skin dip method (excised-shaking). Bacterial count determined by rinsing the whole carcass did not directly relate to counts determined using the skin maceration method. A template for removing a determined meat surface was used by Yokoya and Zulzke (1975). A stainless steel plate with an oval hole in the center was pressed on the meat surface and a sterile knife is used to cut the exposed area on the carcass surface in one movement. This technique also showed higher counts than the cotton swab method on beef carcasses. Its simplicity, rapidity, and adaptability are suitable for routine use on any type of carcass. A coring device was developed by Emswiler et al. (1978) for sampling meat surfaces. Aerobic plate counts of the surfaces of beef and pork carcasses were significantly higher when determined by the coring method than by the cotton or

alginate swab technique. Since cleaning and sterilizing the corer between samplings were time-consuming, it may not be appropriate for routine quality control purposes in the meat industry. The major disadvantage of the destructive method is that it lowers the carcass value, which would be an important consideration if routine quality control analyses are to be made.

III. Rapid Assessment of Microbial Contamination on Surface

Although many techniques have been proposed for microbiological sampling of meat surfaces, very few have received widespread practical application. While the most widely accepted methods are not the most efficient in their removal of microbial cells, they appear to have common characteristics in that they are simple, inexpensive, and allow rapid sampling with a minimum of personnel and skill (Goulet et al., 1983). A number of attempts have been made to produce sampling devices fulfilling those characteristics as well as obtaining reliable microbial estimation.

1. Methods related to adhesive tape

Acetate film adhesive tape described by Edwards and Hartman (1952), is a contact tool devised to detect and identify molds present on infected surfaces. After the tape is pressed against the surface, the exposed tape is stained and observed microscopically. Material from human cases and from plant leaves has been collected by this method.

A plastic replica-embedding method utilizing fingernail polish to form a peelable film has been devised (Masurovsky and Jordan, 1960). The polish placed on the test surface formed a film that can be removed and gram stained for microscopic examination. The method does not distinguish between living and dead cells.

Ulrich (1964) reviewed several different qualitative and quantitative techniques to remove microorganisms from skin. The tape stripping technique is most valuable in indicating the distribution of bacteria on the skin. A pressure sensitive tape was applied to the skin, removed and either covered with agar in a petri plate or placed on a prepoured plate. Each strip removed a single layer of skin cells and the adhering bacteria. As many as 14 strippings have been performed.

A simple and rapid procedure for sampling the microflora of the skin surface by means of an adhesive film distributed evenly on a small plastic disc was described by Kooyman and Simons (1965). The sticky disc procedure has proved to be a simple and rapid way to sample the bacterial flora of large groups of experimental subjects. It gives direct information regarding the distribution and localization of the organisms on the skin surface. Repeated strippings from an area indicate the depth at which the organisms are found.

Milne and Barnetson (1971) compared skin strippings by vinyl adhesive tape (Scotch tape) and conventional skin scrapings (with a scalpel blade) and found that the vinyl adhesive tape gave satisfactory results.

Although use of adhesive tape for sampling microorganisms from human skin has been reported, this method of sampling microorganisms from meat surfaces has not yet been extensively studied. Fung et al. (1980) found that the tape method, especially with mylar adhesive tape, provided a reliable estimate of the microbial load on surfaces of red meat. When being compared with the conventional blend rinse method, the correlation coefficient between 20 paired psychrotrophic counts was 0.95 and 20 paired mesophilic counts was 0.90.

A bacterial detection unit, CON-TACT-IT, based on the tape contact concept, was developed commercially (Birko Corporation, Westminster, Colorado). The sterile sample film (3/4 square inch) was pressed onto the tested surface and

immediate transferred to a square of agar in the special agar plate subdivided into 9 numerically identifiable square areas. After incubation for 18-48 hr at 35°C, number and kinds of bacteria on the plates can be evaluated visually. The CON-TACT-IT technique was compared with the cotton swab method. The swab method had a medium recovery and average precision whereas CON-TACT-IT showed a lower recovery but high precision. A strong correlation was shown between CON-TACT-IT and the swab method when the surface was heavily contaminated (i.e. between 10-100 colonies per sampling area) but the correlation decreased when surfaces contained 10 colonies or less per sampling area. Investigators from the University of California at Davis and Texas A & M University suggested that this commercial tape method was not applicable on surfaces containing completely dried organic soils but it can be used for monitoring plant sanitation if some means of evaluating the results is included in the program. The operators should establish guidelines for each surface to be tested.

2. Swab/agar slant method

Hansen (1962) developed a swab/agar method by streaking the charged swab directly on to agar slants. The slants were grouped according to the number of colonies formed during incubation, and the average number of colonies and its standard deviation were determined by plotting the distribution on probability paper. The method is particularly well suited for large scale field examination.

3. Swab/agar plate method

A labor-saving method combining template and swabbing techniques for sampling the surface of meat was developed by Ølgaard (1977). Samples from swabs are streaked directly on segments of an agar plate. Growth on agar can be

compared with a control comparator disc and segments are given "points value" for different microbial loads. The average "points" from similar sites of carcasses can be obtained and the number of bacteria can be ascertained by use of a conversion table. Ølgaard (1977) used this method to study surface bacteria of beef, pig, bacon and boned meats. This method can only provide limited bacterial ranges.

4. Short-time membrane filter method

Frazier and Gneiser (1968) used a membrane filter method for estimation of numbers of viable bacteria in rinsings or swabbings from the surfaces of foods or equipment in 8 hr. After a measured volume of culture suspension or liquid sample was filtered through a membrane filter it was transferred onto an absorbent pad saturated with trypticase soy broth. After incubating for 8 hr at 35°C, the filter was removed and stained. Colonies were counted with the aid of a microscope at 150x magnification. Results from dairy rinses, fresh green beans and sweet corn by the membrane filter method were comparable to the conventional standard plate count method.

Winter et al. (1971) also used a similar method to study rinsings from food or swab samples. The filters were incubated on a suitable medium for 4 hr at 30°C, heated, stained, and examined microscopically under oil immersion. A scale was used for recording counts and a correlation coefficient of 0.906 was obtained between standard plate count method and this method.

5. Agar sausage method

Ten Cate (1965) developed an agar sausage method which was rapid and simple for the bacteriological examination of contaminated surfaces. It was a contact method where culture media were prepared in artificial sausage casings

to press against the surface to be sampled. The results were determined according to six levels of contamination for making comparisons. It was possible to obtain a fairly objective picture of the conditions in a factory and of the efficacy of the cleaning and disinfection.

6. Rapid contact unit

An easy-to-use testing method, available commercially (Technical Products Division of MCE, Chester, NJ), is based on the slide culture technique in which a plastic is covered on both sides with growth medium, and enclosed in a sterile vial. After the slide has been incubated, a semi-quantitative test is made by simply comparing the bacterial colony density on the growth medium with the density on a model chart provided by the manufacturer. The company claimed that the method provided reliable, economical and time-saving monitoring of microbiological hygiene.

7. Resazurin reduction measurement

A resazurin reduction test was designed to assess the bacteriological quality of meat. A meat sample was weighed and massaged in Stomacher® with sterile diluent for 30 sec. The time required for one ml of the supernatant liquid in the Stomacher® bag mixed with resazurin to change from the blue color to the purple pink color (according to Munsell color standards) was recorded. Holley et al. (1977) tested a variety of fresh and frozen samples of beef and pork, and obtained an apparent relationship between reduction time and results of the standard plate count. Dodsworth and Kempton (1977) established a correlation ($r = -0.93$) between reduction time and bacterial numbers. They suggested that the resazurin test was applicable to all meat cuts purchased in plastic-film bags to which air has access; whether beef or pork in either fresh or frozen state.

Spoiled meat (more than 10^7 bacteria/g) can be detected within 2 hr and the quantitative assessment was superior to sensory evaluation.

The Mechanism of Attachment of Bacteria to Meat Surfaces

Different testing procedures exert different forces upon the surface of meats and thus most likely remove microorganisms at different rates. In addition, the removal and subsequent recovery of microorganisms from meat and poultry surfaces by any of the testing procedures will depend on the forces by which the microorganisms are held in or onto the meat. An understanding of this variable requires information about the mechanism of attachment onto and detachment of bacteria from meat surfaces. Meat surfaces, especially immediately after slaughter, are ideally-suited for bacterial attachment and growth. An attachment to the meat surface may be considered a first step in the microbial spoilage of meat.

I. The Attachment Process

The process of attachment is rather complicated and perhaps the mechanism of attachment involves two consecutive stages. In the primary stage, reversible sorption, bacteria are attracted to and held weakly near the surfaces. Marshall et al. (1971) found this stage to be dependent upon the electrolyte concentration, and they suggested that the reversible sorption is associated with the Van der Waals attraction energies between two surfaces and the electrical repulsive energies resulting from overlapping ionic atmospheres around the surface. The bacterial attachment is due to physical forces, and the number of bacteria attached is proportional to the number in the water film surrounding the surface. The second stage, irreversible sorption, is initially characterized by an

increased strength of attachment due to polysaccharide formation, which is somewhat time dependent. This irreversible phase of sorption implies a firmer adhesion of bacteria to a surface. Polymeric bridging between the bacterial surfaces and that of the test surface might overcome the repulsion barrier between such surfaces (Firstenberg-Eden, 1981; Marshall et al., 1971). Fletcher and Floodgate (1973) demonstrated an acidic polysaccharide layer which was involved in adhesion of a marine bacterium to surfaces. In the attachment of bacteria to mammalian cells, it is possible that after the first stage of weak reversible adhesion there is an intermediate stage of firmer adhesion by fimbriae and pili, involving formation of hydrogen or ionic bonds, and this is followed, as with the marine bacteria, by formation of extracellular polysaccharides (Firstenberg-Eden, 1981). Corpe (1970) reported the production of an extracellular acid polysaccharide by a primary film-forming bacterium, Pseudomonas atlantica. Glass slides coated with this polymer became fouled with microorganisms more rapidly than uncoated slides. Costerton et al. (1978) claimed that bacteria become attached in nature by means of a mass of tangled fibers of polysaccharides or branching sugar molecules that extend from the bacterial surface and form a "glycocalyx" surrounding an individual cell or colony of cells. The adhesion mediated by the glycocalyx determines particular locations of bacteria in most natural environments; more specifically, it is a major determinant in the initiation and progression of bacterial diseases. Glycocalyx is chemically defined by the composition of its own particular polysaccharides. The polysaccharide fibers of the bacterial glycocalyx, which are for the most part negatively charged, can form a polar bond with a higher-cell polysaccharide by way of divalent positive ions in the medium. Lectins with a specific attraction for the glycocalyx fibers and for the higher-cell polysaccharides can also form a bridge between them. Bacteria whose fibers can bind neither directly to those of

the higher cell nor to a suitable lectin in the system simply do not adhere. The fibers of the glycocalyx may not only position bacteria but also conserve and concentrate the digestive enzymes released by the bacteria and direct them against the host cell. A glycocalyx can also function as a food reservoir for bacteria. In nature, bacteria are subjected to many sources of stress against which the glycocalyx offers protection.

II. Factors Affecting Attachment of Bacteria to Meat Surfaces

The nature and rate of attachment depend on various factors including the bacteria concerned (concentration, species, growth medium, etc.) and the conditions under which attachment occurs (pH, temperature, contact time, etc.). The surface structure also has a critical influence on the attachment of bacteria.

1. Effect of concentration of bacteria in attachment medium on attachment

Most investigators (Butler et al., 1979; McMeekin and Thomas, 1978; Notermans and Kampelmacher, 1974) agree that a direct relationship exists between numbers of test organisms in the attachment medium and the extent of bacterial attachment; i.e., the higher the initial population, the greater the attachment. McMeekin and Thomas (1978) studied retention of bacteria on chicken skin after immersion in bacterial suspensions, and suggested that the population density of the suspension is the major factor determining the number of bacteria retained on chicken skin immediately after immersion. Therefore from a practical point of view it is important to prevent a build up of contamination at any point in the immersion chilling system used in many poultry processing plants.

Attachment rate is defined as the number of bacteria that attach to a

certain surface area of meat per min, based on the concentration of microorganisms/ml attachment suspension. In an attempt to evaluate the strength with which bacteria remain attached to meat surfaces, Firstenberg-Eden et al. (1978) introduced a parameter to measure the attachment strength or ease of removal of bacteria from the surface of meat. The difference between $\log_{10}$ of the counts obtained by the blend method and the rinse method was called S-value. An S-value of zero would imply that bacteria are loosely attached, while a high S-value would indicate strong bacterial attachment.

2. Effect of bacterial strain and meat surface

The kinetics of attachment also depend on the bacterial strain. Firstenberg-Eden et al. (1978) found that some bacteria are better able to attach to surfaces than others. From their study of attachment of certain bacterial strains to different meat surfaces, Pseudomonas EBT/2/143 had the highest attachment rate for all six surfaces investigated. Butler et al. (1979) also found that Pseudomonas strains attach to meat surfaces more rapidly than do other bacteria. Farber and Idziak (1984) studied attachment of psychrotrophic meat spoilage bacteria to muscle surfaces, and concluded that Pseudomonas fluorescens and Brochothrix thermosphacta were best suited for attachment and adherence to meat surface; the nonfluorescent pseudomonad was the least suited for attachment.

There is a debate on the role and importance of flagella in the attachment process. Notermans and Kampelmacher (1974) suggested that motile bacteria were retained more readily on chicken skin than non-motile bacteria and suggested the accumulation of motile organisms might be the result of flagella activity and chemotaxis. Butler et al. (1979) investigated bacterial attachment to pork skin and surfaces of beef and lamb carcasses, and found that attachment of motile

gram-negative species (Escherichia coli, Pseudomonas putrefaciens, Erwinia herbicola) was greater than that of non-motile, gram-positive organisms (Lactobacillus, Staphylococcus). Farber and Idziak (1984) indicated that the lowest attachment values were recorded with two non-motile bacteria, Acinetobacter LD-2 and Moraxella osloensis; the highest, with two motile fluorescent pseudomonads, Pseudomonas putida and Pseudomonas fluorescens. The non-motile bacteria possessed higher S-values than the motile organisms. Flagella contribute to an active transport of the bacteria to the surface, which may increase the attachment rate (Firstenberg-Eden, 1981). However, McMeekin and Thomas (1978) believe that motility had a negligible effect on the number of organisms retained on the chicken skin. Their result was in agreement with the data of some other researchers which indicated that the flora of chicken carcasses immediately after processing comprised >70% of non-motile types (McMeekin and Thomas, 1978).

The nature of surface structure has a direct effect on bacterial attachment. Firstenberg-Eden et al. (1978) studied the attachment of bacteria to chicken and beef meat with and without fascia, and concluded that chicken breast with fascia was the best surface for the attachment of all the bacteria examined. It is possible that bacteria which attached well to one surface may attach more slowly to a different surface.

3. Effect of time, temperature, and pH on attachment

Firstenberg-Eden et al. (1978) stated that a linear relation between the number of bacteria attached and time was found at the beginning of the attachment process. On some surfaces the linear relationship continued for a long time (teats of cow, chicken meat with fascia). However, on other surfaces the attachment stopped after 25-35 min (cut chicken meat, cut beef meat, etc.).

In some cases extraction of proteins from the meat was observed and this could be the reason for the attachment stopping. McMeekin and Thomas (1978) found that while Pseudomonas sp. had a linear relationship between numbers of attached bacteria and time, the number of Escherichia coli and Micrococcus sp. attached did not increase with time after the first minute of immersion. In the study of effect on the S-value of extended contact of the bacteria with meat, Firstenberg-Eden et al. (1978) reported that the S-value for Salmonella infantis was lower than that for either E. coli or P. fluorescens. As time of contact increased, the S-value for S. infantis increased to a greater extent than that of the two motile organisms.

Butler et al. (1979) investigated effect of temperature on attachment of bacteria onto pork skin, and found that with E. coli, P. putrefaciens, and the Lactobacillus and Staphylococcus spp. little difference in attachment occurred over a wide range of temperature (2.5-37°C) of the attachment medium. With Erwinia herbicola, attachment was greater at temperatures ranging from 26 to 37°C than at the lower temperatures (3-7.5°C). Notermans and Kampelmacher (1974) reported that temperature affects the attachment rate, the optimum being around 21°C. The increase in the rate from 0°C to 21°C may be explained by an increased activity of the microorganisms combined with the flagella activity. A decrease in attachment rate at temperatures above 21°C may be due to the increased activity of flagella by which bacteria become free from the skin.

When the pH of the attachment medium was adjusted to values ranging from 4.45-9.3, optimum attachment for bacteria occurred at about pH 8.0 (Vanderzant et al., 1976). According to Notermans and Kampelmacher (1974), the effect of lowering the pH of the attachment medium on the rate of attachment is related to a decrease in bacterial mobility. Butler et al. (1979) found that no particular pH value seemed to favor attachment of all five organisms tested. Notermans and

Kampelmacher (1983) suggested that changing the pH to 4.5 or lowering the temperature had little effect on attachment. The overall effect of lowering temperature, pH and adding Tween-80 and mannose on the reduction of Attachment was 10-100 fold. From these results it can be concluded that bacterial species do not have the same mechanisms of attachment to meat surfaces. Therefore, attachment can only partially be prevented by adding chemical substances and by changing physical conditions. In practice, this will not result in a complete reduction in the bacterial count of the final product.

III. Secondary Attachment During Storage

In these experiments (Firstenberg-Eden, 1981; Firstenberg-Eden et al., 1978; Firstenburg-Eden et al., 1979), the bacteria were allowed to attach to the surface from a bacterial suspension (at 20°C for 20 min). After washing the water film, the meat was stored at 20°C. The number of bacteria present after different storage times was estimated, using both the blend and the rinse methods. The results for teats of cows showed that the S-value increased during 1-3 hr of storage, although the actual number of bacteria increased only moderately during this time. Longer storage times resulted in a decrease in S-value, and at the same time the bacterial counts in both methods increased (Firstenberg-Eden, 1981). To explain those results and learn more about the mechanism of secondary attachment, scanning electron microscopy (SEM) was used (Firstenberg-Eden et al., 1979). Teats of cows were artificially contaminated with Pseudomonas EBT/2/143. No production of extracellular material could be seen after the primary attachment. After 3 hr at 20°C production of extracellular polymers in the form of thin fibers could be observed. The numbers of those fibers and their thickness increased during storage. By 12 hr, slime had been produced, and microcolonies were observed. During secondary

attachment, production of extracellular polymers was observed for all bacteria examined. The different amounts of slime found on the microcolonies were probably due to differences in available nutrients. Zobell (1943) stated that slime production by attached bacteria appeared to be influenced mainly by the micro-ecosystem. He found that more slime was produced in ecosystems in which it was difficult for bacteria to survive. The increase in S-value during the first 2-3 hr of storage could be explained by formation of extracellular fibers. The decrease in strength of attachment found after 12 hr of storage was probably due to formation of easily removable microcolonies in which more and more bacteria were attached to each other and not directly to the skin.

IV. Potential Consequences of Attachment

The existence of specific mechanisms of attachment emphasizes that attachment is a function of the physical parameters and biological properties of the bacteria. Although the mechanisms of bacterial attachment to meat surfaces are not well understood, the practical consequences are clear. First, if a bacterium makes contact with a meat surface, attachment will occur. Although the rate of attachment may be influenced by several external factors, a zero attachment will never be achieved. Secondly, bacteria, once attached to a surface, are difficult to remove, also attached bacteria are protected against stresses like disinfectants, heat treatment and gamma irradiation (Notermans and Kampelmacher, 1983). With respect to food plant hygiene, attachment permits bacteria present in low numbers in water surrounding meat to become concentrated on the meat surface. While the bacteria are in the water film, they may still be easily removed. With time, these bacteria could attach to the meat and become difficult to remove. However, attachment, with resulting contamination peaks, could be prevented by continuous spray cleaning (Notermans

and Kampelmacher, 1975a). Pathogenic bacteria are potentially dangerous to the consumer, but when strongly attached may be less dangerous, as far as kitchen and equipment hygiene is concerned, than the bacteria that are only weakly attached. The latter are more likely to be able to spread and they may also be potentially more dangerous to the consumer as they can contaminate surfaces, utensils and hands. Firmly attached bacteria will not contaminate surfaces when meat is thawed since the attached bacteria are not easily transferred to the drip water, and will normally be killed during the cooking process (Firstenberg-Eden, 1981; Firstenberg-Eden et al., 1978).

Off-Odor as Related to Microbial Contamination on Meat

Growth of bacteria on meat results in the production of off odors and flavors or an appearance unacceptable to the consumer. Microbial spoilage is delayed, but not prevented, by storage of meat at temperatures between -1° and +5°C. The shelf life of raw meat stored under chill conditions will depend upon the interaction of several intrinsic and extrinsic factors. These include the numbers and types of spoilage organisms present initially, the storage temperature, muscle type and pH, type of packaging material used and gaseous environment of the product. During refrigerated storage there is a marked change in the relative proportion of the different psychrotrophs present and at spoilage the predominant organisms are usually pseudomonads with somewhat lower levels of Acinetobacter-Moraxella spp. and Alteromonas putrefaciens. Off-odors are detectable when the total count at 1 or 20°C reaches ca. 10^8 organisms/cm^2 and appear to be due to a variety of volatile compounds formed during bacterial growth (Mead, 1982). Development of strong off-odors characteristic of spoiling flesh foods at chill temperatures occurs as a result of

the growth and metabolism of a restricted group of psychrophiles (Daud et al., 1979 ; Herbert et al., 1971; McMeekin, 1975). McMeekin (1977) found that the incidence of off-odor producers, expressed as a percentage of the psychrophilic flora, remained uniformly low throughout the storage period, with a maximum of 21% off-odor producers recorded after 16 days of storage. Patterson (1972) pointed out that a mixed flora were isolated at the spoilage stage and it is probable that no one particular group of organisms was responsible for the off-odors, rather that some strains attacked the available proteinaceous substrates while others attacked the chicken fat.

I. The Microorganisms Involved

Strains of Pseudomonas, Moraxella, Acinetobacter, Lactobacillus, Microbacterium thermosphactum and certain genera of the family Enterobacteriaceae are the most common bacterial types found on spoiled meat. Jensen (1944) reported that Pseudomonas was the chief organism that caused spoilage of refrigerated meat. Gill and Newton (1977) found that pseudomonads grew faster than the other species at all temperatures between 2° and 15°C. These organisms reproduce rapidly and cause a typically rancid, sweetly aromatic esterlike "dirty dishrag" odor when dressed chickens are stored long enough at refrigeration temperatures and high humidity. At the time birds develop off-odor or become slimy, Pseudomonas and Achromobacter spp. account for 90% or more of the total population (Ayres et al., 1980). Barnes and Melton (cited by Patterson, 1972) have examined the extracellular activity of strains isolated mainly at 1°C from spoiling chickens and found that proteolytic activity was mainly among two groups of pigmented pseudomonads and P. putrefaciens. The ability to attack chicken fat was particularly evident among the non-pigmented pseudomonads. Cox et al. (1975) compared the differences between

non-pigmented and pigmented Pseudomonas and noted that non-pigmented strains produced more intense off-flavor in poultry meat. Pseudomonas group I and II types developed most rapidly on spoiling chicken leg muscle stored at 2°C and eventually dominated the spoilage association. Alteromonas putrefaciens remained a consistantly small fraction of the flora but produced strong off-odors when grown on chicken leg muscle (McMeekin, 1977).

II. Types of Off-Odors

Volatile sulfides in low concentrations probably contribute to the flavor of fish as they do to most proteinaceous foods such as chicken, beef, and milk whereas at higher concentrations their objectionable properties are detrimental to the flavor of such foods (Herbert and Shewan, 1975). The organism most commonly responsible for sulfide-like off-odors has been Alteromonas putrefaciens, which produces hydrogen sulfide, methyl mercaptan, and dimethyl sulfide by degradation of the sulfur-containing amino acids cysteine and methionine (McMeekin, 1977). Sulfide-like odors were also produced on chicken leg muscle by some fluorescent pseudomonads, and this property has been recorded for Pseudomonas fluorescens growing on fish (McMeekin, 1977). Three distinctive types of odor were recognized in spoilage of chicken breast muscle (McMeekin, 1975). Sulfide-like odors by Pseudomonas type IV rose to a peak (22% of samples) with the strains isolated after 8 days of storage, fruity types remained uniformly low throughout, and strains giving the evaporated-milk odor increased steadily during storage, but particularly rapidly between the 12- and 16-day stages. The predominant odor produced by the Pseudomonas II organisms was the evaporated-milk type. Some Pseudomonas group II strains produced the fruity-ester-like odors described for spoiling fish, fish muscle and chicken breast muscle inoculated with Pseudomonas fragi (McMeekin, 1977). Igbinedion et al.

(1983) studied effects of storage time on microbial growth and rancidity of fresh pork and found that a significant ($P < 0.01$) linear response was evident when relating storage time to rancid flavor. As storage time increased, rancid flavor became more pronounced. Thornely et al. (1960) reported that a putrid, ammonia-like odor appears when non-pigmented Pseudomonas reaches a level exceeding $10^7/cm^2$ of skin.

III. Bacterial Spoilage Mechanism in Meat

Until spoilage is evident to the senses, the only detectable effect of bacterial growth is some reduction of the glucose concentration, which does not alter the organoleptic qualities of the meat. However, on exhaustion of glucose more obvious and drastic changes occur. Spoilage odors and flavors are due to the production of volatile substances as a result of microbial degradation of amino acids (Gill, 1982). Low molecular weight compounds of amino acids and nucleotides rather than primary beef proteins, support the growth of beef spoilage bacteria (Jay and Kenton, 1967). The foul odors were believed to have come from degradation of these low molecular weight compounds which formed ammonia, hydrogen sulfide, indol and other foul-odor compounds. Gill and Newton (1980) found that bacterial spoilage of adipose tissue and that of muscle tissue are essentially similar. In both cases spoilage occurs as a result of degradation of amino acids. Adipose tissue contains low molecular weight soluble substances which are present in low concentration at the surface of adipose tissue and are utilized in preference to lipid for bacterial growth. Bacteria growing on a thin layer of agar over an adipose tissue surface utilized glucose preferentially. Upon the disappearance of glucose, amino acids were attacked, producing malodorous substances which were detectable as spoilage odors when the cell density was about $10^6/cm^2$. In normal muscle tissue, the bacteria must reach a cell density in

excess of $10^8/cm^2$ before glucose is exhausted at the meat surface and amino acids are attacked. Under humid conditions, aerobic flora are usually dominated by pseudomonads while anaerobic flora are dominated by lactobacilli. In both cases growth occurs on low molecular weight soluble components of meat. The order of attack is first glucose, then glucose-6-phosphate and amino acids. Under aerobic conditions spoilage becomes detectable when the bacteria begin to degrade amino acids which remain abundant at the meat surface when growth ceases, probably because of limited availability of oxygen. Under anaerobic conditions growth ceases because the diffusion of fermentable substrates to the surface is not rapid enough to support further growth. Aerobically, there is no interaction between different bacterial species until the maximum cell density is approached; anaerobically, lactobacilli produce an antimicrobial agent which inhibits growth of competing species (Gill and Newton, 1978).

MATERIALS AND METHODS

Source of Meat Samples

Retail raw meats packed in polyvinyl chloride (PVC) packages, were purchased from local grocery stores. Chicken samples included chicken wings, drum sticks, breasts, and frying pieces. Pork samples included pork chops, pork loin chops, and ribs.

Cured meat products included bacon, bone-in hams, and boneless hams, were sampled from the products displayed in a local annual professional convention.

Surface Microbial Sampling Methods

I. Tape Method

1. The procedure of tape sampling

Mylar adhesive tape (Dynatech Laboratory Inc., Alexandria, VA) with adhesive area of 2 X 4 cm was cut for surface sampling. After peeling the protective paper from the tape, the adhesive side was pressed on the surface of meat sample. The adhesive side of the tape has been determined to be free of microorganisms by Fung et al. (1980). After 1 min contact time, the tape charged with microorganisms was then transferred to sterile plate count agar (Difco) contained in a round petri dish (100 X 15 mm). After contact time of 1 min on agar surface, the tape was removed and the plate was incubated. Another tape was employed to the meat surface adjacent to the first tape sampling area. Then, the tape was transferred to sterile brilliant green agar (Difco) surface as previously described. Plate count agar plates were incubated at 32°C for 24 hr for mesophilic counts

and brilliant green agar plates were incubated at 37°C for 24 hr for gram-negative bacterial counts.

2. Enumeration of microorganisms from tape method

After incubation, the number of colonies on the plate was counted directly when numbers were fewer than 500 per plate, and was converted into log CFU (colony forming units)/cm^2. When larger numbers were encountered, a template with 8 squares was used to estimate the number of colonies on the plate. Each square possesses 1 cm^2 area. The number of organisms in 4 squares was counted. The total number in 4 squares was multiplied by 2 to obtain the total estimated number of CFU/cm^2 on a plate. When the organisms in a square can not be counted individually, it was recorded as too numerous to count, an arbitrary "saturation" number of 200 colonies was assigned. This tape counting procedure was made after 8, 16, or 24 hr to ascertain the shortest possible time for making viable cell count.

3. Estimation of microbial load by staining of adhesive tape

This staining technique was designed to estimate microbial load on meat surface at 0 time as well as growth of these organisms after 4 hr incubation on agar. After 1 min contact time on meat surface, the tape was impressed on agar surface for another 1 min. The tape was then removed from the agar and the conventional gram staining procedure was made on the tape. The stained tape was observed under oil immersion. This gives the 0 time direct tape count. After incubating the agar plate for 4 hr, a new tape was pressed on the agar surface exactly on the position which was previously charged with sample. After contact time of 1 min, the new tape was removed, gram stained, and observed under oil immersion for the 4 hr direct tape count. To facilitate accurate sampling

between the 0 time and 4 hr, the Rodac® plates (65 X 15 mm, Div. Becton, Dickinson & Co., Oxnard, CA) with 10 mm grids were used. When enumerating colonies on the tape under oil immersion microscopy, 10 predesignated fields were counted. The fields were located by predetermined vertical and horizontal axes on the stage of a microscope. The total microbial distribution in 10 fields determined microbial load on the tape and the level of contamination on the test surface sample. The scales used to estimate the number of microorganisms on the tape are as followed:

Very Low Count (VL): total counting of 10 fields ranging from 0 to 20.

Low Count (L): total counting of 10 fields ranging from 30 to 200.

Intermediate Count (I): the average count of 10 fields ranging from 30 to 300.

High Count (H): number of colonies within the field is uncountable; bacteria distributing in about half of one field, and over 5 out of 10 fields are recorded as high count.

Very High Count (VH): number of colonies within the field is uncountable; bacteria growth in one field is about full, and over 5 out of 10 fields are recorded as very high count.

On some occasions the counts fell between Very Low and Low (VL-L) or Low and Intermediate (L-I). VL-L is defined as total counting of 10 fields is 20 to 30, and L-I is defined as total counting of 10 fields is 200 to 300.

II. Swab Method

Guided by a sterile template cut out of aluminum foil, a moist sterile cotton swab (American Scientific Products. McGaw Park, IL) was rubbed over a meat surface area of 2 X 4 cm in three different directions. The area is juxtaposed to the tape area on the same meat sample. The cotton swab was then aseptically

broken into a tube containing 2 ml sterilized phosphate buffer solution. After shaking the tube containing the swab for 40 times, the standard plate count procedure was used to obtain viable cell count of the surface of meat in cm^2. Incubation time was 24 hr at 32°C for total bacterial count and 37°C for gram-negative bacterial count.

III. Excised-Blending Method

Sterile scalpel and forceps were used to excise a piece of surface meat (2 X 4 cm) close to the tape sampling area aseptically. The excised meat sample was put into a Stomacher® '400' bag (Dynatech Laboratory Inc., Alexandria, VA) containing sterile 100 ml phosphate buffer. The Stomacher bag was then massaged in the Stomacher Lab Blender 400 for 1 min. Serial dilutions were made from the blended sample solution and spread plate technique was applied to each different level of dilution with duplicate agar plates. After incubation, the number of colonies on the plates was counted and recorded as log CFU/cm^2 When data were expressed for per gram, the samples were aseptically weighed before blending and the results were expressed as log CFU/g.

Meat Odor Associated with Microbial Growth on Surface

I. Meat Samples

Meat samples (chicken and pork) with varying display dates were obtained from local grocery stores to ensure a variety of contamination levels. Individually wrapped meat samples were stored at 7°C before odor evaluation and microbial load estimation.

II. Odor Evaluation

Six panelists from the Animal Sciences and Industry Department, KSU, evaluated meat odor by sniffing the samples at 0 day and after 3 days at 7°C. Panelists were given a fresh meat and a poor quality meat as index of odor before each experiment. A short training period was held before each test. The odor scale used was as follows: 1- fresh (no off-odor); 2- slight off-odor; 3- moderate off-odor; 4- strong off-odor; 5- extreme off-odor (D.H. Kropf and M.C. Hunt, Department of Animal Sciences and Industry, KSU. personal communication). The scale was based on the intensity of off-odor. Mean and standard deviation of odor scores for each sample from 6 panelists were calculated as an averaging score (Snedecor and Cochran, 1980).

III. Microbial Load Estimation

Microbial loads on all meat surfaces at 0 day and 3 days storage at 7°C were estimated immediately after odor evaluation. The 0 time direct tape count and 4 hr direct tape count were used to estimate surface contamination. The swab method was also applied as a comparison. Sampling (tape method and swab method) of microbial loads on the meat sample was made on different areas of the meat between 0 day and 3 days storage.

RESULTS AND DISCUSSION

The mylar adhesive tape method was developed to estimate surface contamination on different meat samples. The procedure of 24 hr tape-counting method used by Fung et al. (1980) was tested on chicken wing samples and cured meat products. Then, the tape method was improved to reduce the incubation time and obtain reliable, faster results. Furthermore, a staining technique of tape was adapted to estimate the microbial loads on chicken and pork surfaces at 0 time and 4 hrs. Prediction of odor of meat during storage was also evaluated by the 0 time and 4 hr direct tape count procedure.

The Comparison of 24 Hr Tape-Counting Method and Swab Method on Chicken Wing Samples

Results of mesophilic and gram-negative bacterial counts of chicken wing samples by the 24 hr mylar adhesive tape method and the conventional swab method are presented in Figs. 1 and 2. Microbial loads on meat surfaces were log 1-8 CFU/cm^2 by the conventional swab method and log 1-2.3 CFU/cm^2 by the 24 hr tape method. Table 1 shows correlation coefficients between the two methods for 51 paired mesophilic counts was 0.77 and for 47 paired gram-negative bacterial counts was 0.83 (Snedecor and Cochran, 1980). When the tape count was >log 2 CFU/cm^2, conventional bacterial counts (both mesophilic and gram-negative) were high on chicken meat surfaces (ca. log 5-8 CFU/cm^2) and when the tape count was <log 2 CFU/cm^2, conventional counts were <log 5 CFU/cm^2. When the tape count reaches saturation point (ca. log 2.3 CFU/cm^2), conventional bacterial counts reach log 6 CFU/cm^2 or higher. These data indicate that the tape method is a convenient and simple method for estimating

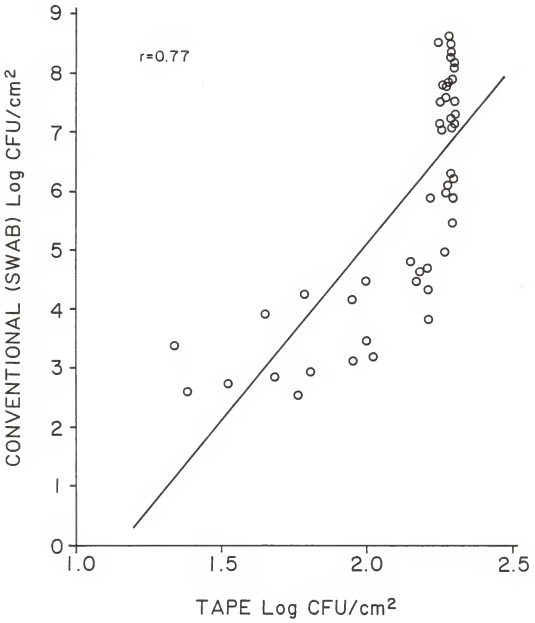

Fig. 1. Mesophilic bacterial count of chicken wing surfaces by swab vs. 24 hr-tape method.

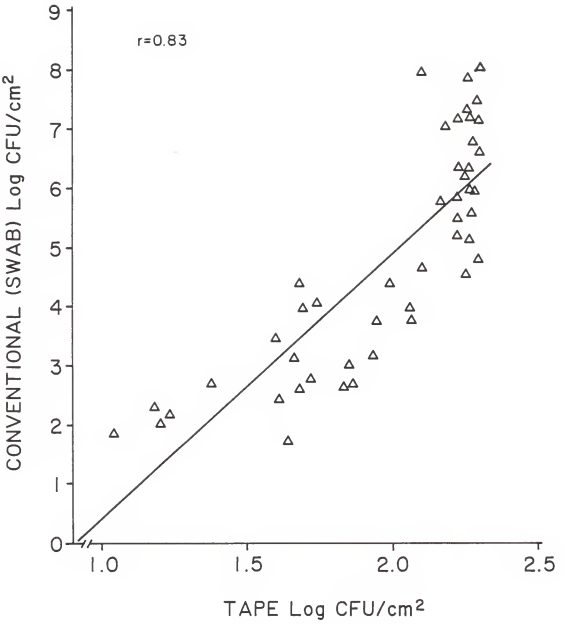

Fig. 2. Gram negative bacteria count of chicken wing surfaces by swab vs. 24 hr-tape method.

Table 1. Correlation coefficients and 95% confidence intervals of correlation coefficients for the comparison of different methods.

	Chicken (Total count)	Chicken (Gram-negative)	Pork	Cured meat
24h tape vs. swab	0.77 (n=51) $0.63 \leq r \leq 0.86$	0.83 (n=47) $0.72 \leq r \leq 0.90$	--- ---	--- ---
24h tape vs. excise	---	---	---	0.85 (n=26) $0.70 \leq r \leq 0.93$
4h tape vs. swab	0.96 (n=16) $0.89 \leq r \leq 0.99$	--- ---	0.93 (n=22) $0.83 \leq r \leq 0.97$	--- ---
0h tape vs. swab	0.82 (n=16) $0.55 \leq r \leq 0.94$	--- ---	0.80 (n=22) $0.57 \leq r \leq 0.91$	--- ---
4h tape vs. excise	0.82 (n=14) $0.55 \leq r \leq 0.94$	--- ---	0.87 (n=22) $0.72 \leq r \leq 0.95$	--- ---
0h tape vs. excise	0.52 (n=14) $-0.01 \leq r \leq 0.82$	--- ---	0.82 (n=22) $0.61 \leq r \leq 0.92$	--- ---
Odor vs. swab (0 day)	0.80 (n=10) $0.35 \leq r \leq 0.95$	--- ---	0.91 (n=10) $0.66 \leq r \leq 0.98$	--- ---
Odor vs. swab (3 days)	0.80 (n=10) $0.34 \leq r \leq 0.95$	--- ---	0.85 (n=10) $0.48 \leq r \leq 0.96$	--- ---
Odor vs. 4h tape (0 day)	0.66 (n=10) $0.05 \leq r \leq 0.91$	--- ---	0.76 (n=10) $0.25 \leq r \leq 0.94$	--- ---
Odor vs. 0h tape (0 day)	0.78 (n=10) $0.30 \leq r \leq 0.95$	--- ---	0.87 (n=10) $0.53 \leq r \leq 0.97$	--- ---
Odor vs. 4h tape (3 days)	0.67 (n=10) $0.07 \leq r \leq 0.91$	--- ---	0.07 (n=10) $-0.59 \leq r \leq 0.67$	--- ---
Odor vs. 0h tape (3 days)	0.89 (n=10) $0.59 \leq r \leq 0.97$	--- ---	0.40 (n=10) $-0.30 \leq r \leq 0.82$	--- ---

contamination level of meat samples.

The Comparison of 24 Hr Tape-Counting Method and Excised-Blending Method on Cured Meat Products

Results of total surface bacterial count of 26 cured meat product samples by 24 hr adhesive tape method and the conventional excised-blending method are shown in Fig. 3. Microbial loads were log 1-6 CFU/cm^2 by the excised-blending method and log -1 to 2 CFU/cm^2 by the 24 hr tape method. Correlation coefficient between the tape method and conventional method was 0.85 when using surface count (log CFU/cm^2) for comparison in the conventional method. These data indicate that when the tape count is >log 2 CFU/cm^2, surface bacterial counts are high on meat surfaces (ca. log 5-7 CFU/cm^2); between log 0.5-1.5 CFU/cm^2, counts are intermediate (ca. log 3-4 CFU/cm^2) and <log 0.5 CFU/cm^2, conventional counts are low (ca. <log 3 CFU/cm^2). These results are similar to data obtained from beef samples by Fung et al. (1980). They suggested that when the tape count is >log 2 CFU/cm^2, bacterial counts are high on meat surfaces (ca. log 5-7 CFU/cm^2); between log 1-2 CFU/cm^2, counts are intermediate (ca. log 3-4 CFU/cm^2) and <log 1 CFU/cm^2, counts are low (ca. log 3 CFU/cm^2). The correlation coefficient (0.85) obtained in this study is lower than that obtained (r = 0.90) by Fung et al. (1980). Since cured boneless ham may have bacteria inside the sample, the data were also expressed as log CFU/g (Fig. 4). When the tape count is log 2 CFU/cm^2 (about saturation point), conventional bacterial counts are high in meat (ca. log 6-7 CFU/g); between log 0.5-1.5 CFU/cm^2, counts are intermediate (ca. log 3.5-5 CFU/g) and <log 0.5 CFU/cm^2, counts are low (ca. <log 3.5 CFU/g). It appeared that bacterial counts of conventional excised-blending method are slightly higher in using the unit of log CFU/g than that of log CFU/cm^2 from all 26 sample surfaces. These data

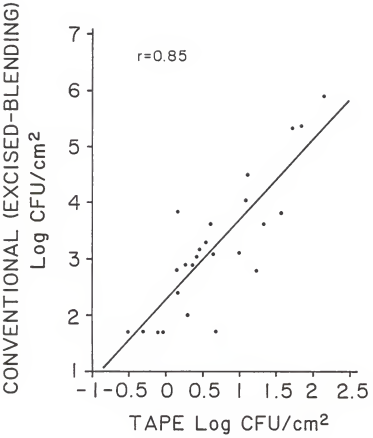

Fig. 3. Practical application from cured meat products. Surface tape count vs. surface area count (cm^2).

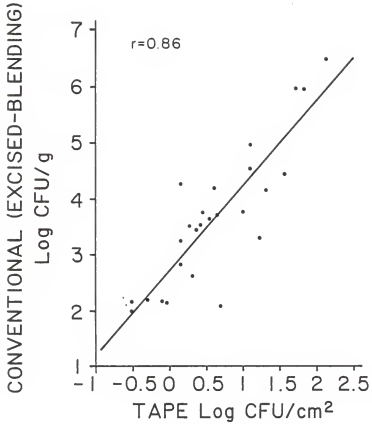

Fig. 4. Practical application from cured meat products. Surface tape count vs. count by weight (per gram).

indicate that the tape method is adaptable not only to raw meat but cured meat samples. Since bacteria distributed evenly in restructured meat compared with raw meat cuts where only surface contamination occurs, the surface tape count actually reflected the quality of the entire product. The data of this experiment indicated that the surface tape count had high correlations with both the log CFU/cm^2 and log CFU/g.

Reduction of Incubation Time for Tape-Counting Method

In the original tape-count method of Fung et al. (1980), 24 to 48 hr incubation time was needed before counting of colonies. However, preliminary experiments indicated that colonies appeared on agar at a much earlier time. It seemed possible to read the tape count earlier to estimate the microbial loads on meats. The agar plates seeded by the tape were examined in 8 hr incubation intervals. Fig. 5 shows that tape counts at 8, 16, and 24 hr provided similar trends in estimating surface count of meat, i.e. regardless of reading times the microbial load on meat surface can be estimated by the tape count thus reducing reading time of tape count from 24 hr to 8 hr.

Estimation of Microbial Loads by Staining of Adhesive Tape

To further reduce reading time of tape count, a new approach was instituted. By use of the microscope one can actually see the microbial population on meat surfaces by staining the adhesive tape after peeling the tape from the meat surface (0 time). In order to determine whether there are viable cells the organisms were allowed to grow on the agar plate for 4 hr before removal by a new tape and observed under the microscope after staining. Any increase in microbial counts would indicate viability of the cells from the meat.

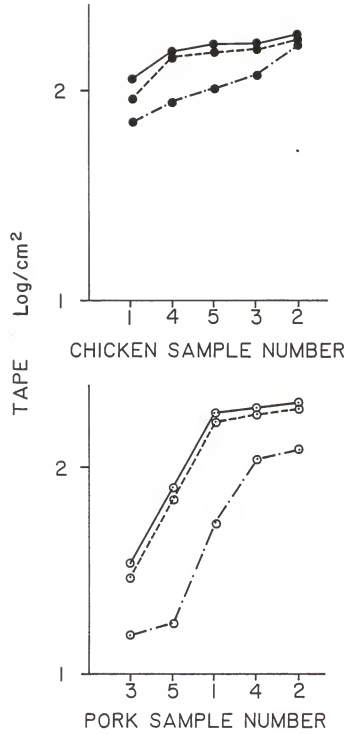

Fig. 5. Colony-counting time (—·—· 8hr, ----16hr, —— 24hr)

Five pork samples were used in the preliminary study of the tape staining technique. Fig. 6 shows that both the 0 time direct tape count and 4 hr direct tape count correlated well with two conventional methods (swab and excise) in terms of microbial loads.

A direct tape count scale was developed for this purpose: very low (VL), low (L), intermediate (I), high (H), and very high (VH).

The Comparison of 0 Hour Direct Tape Count and 4 Hour Direct Tape Count with Two Conventional Methods on Chicken Surfaces

Table 1 shows the comparison between swab method on 16 chicken samples with the 0 hr direct tape count and 4 hr direct tape count. Correlation coefficients between 0 hr direct tape count and swab method was 0.82; whereas the 4 hr direct tape count and swab method was 0.96. Fig. 7 shows that when 4 hr direct tape count is VH (very high), bacterial counts on chicken surfaces by swab method are higher than log 7 CFU/cm^2; when the direct tape count is H (high), bacterial counts are between log 6-7 CFU/cm^2; I (intermediate count), bacterial counts are between log 5-6 CFU/cm^2; L (low count), bacterial counts are between log 4-5 CFU/cm^2 and VL (very low count), bacterial counts are low (ca. log 3 CFU/cm^2).

Tape method was also compared with excised-blending method which correlated better with 4 hr direct tape count ($r = 0.82$) than 0 hr direct tape count ($r = 0.52$). Fig. 8 shows that when 4 hr direct tape count is VH, bacterial counts by excised-blending method are high on chicken surfaces (ca. >log 7 CFU/cm^2); when the direct tape count is H, bacterial counts are between log 6-7 CFU/cm^2; I, bacterial counts are between log 5-6 CFU/cm^2; L, bacterial counts are around log 5 CFU/cm^2 and VL, bacterial counts are around log 4 CFU/cm^2.

Comparing the results of two conventional methods (swab and excise),

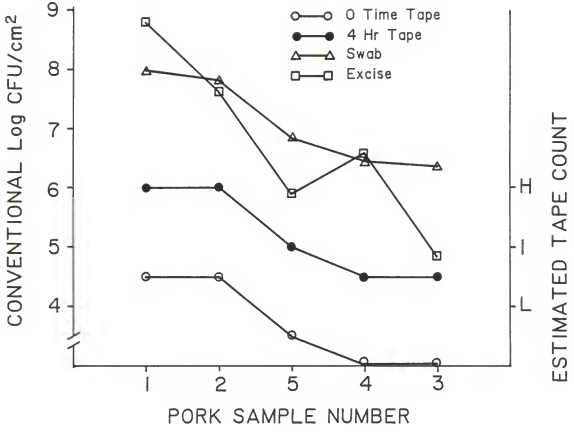

Fig. 6. Tape count compared with swab and excise-blending methods. (L-Low Count; I-Intermediate Count; H-High Count)

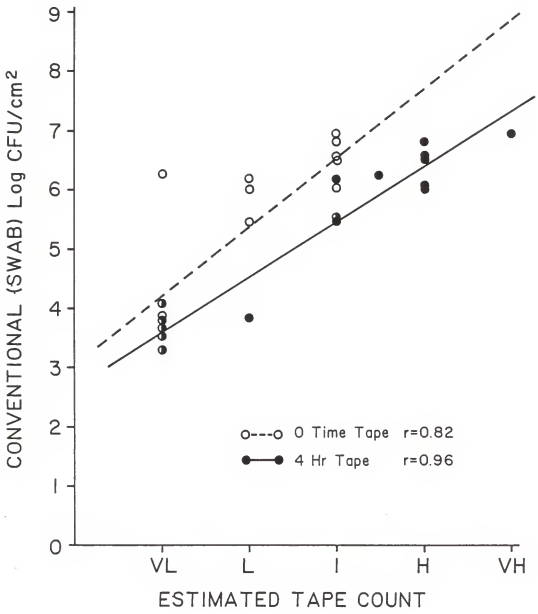

Fig. 7. Surface bacterial count of chicken samples by using tape method vs. swab method.

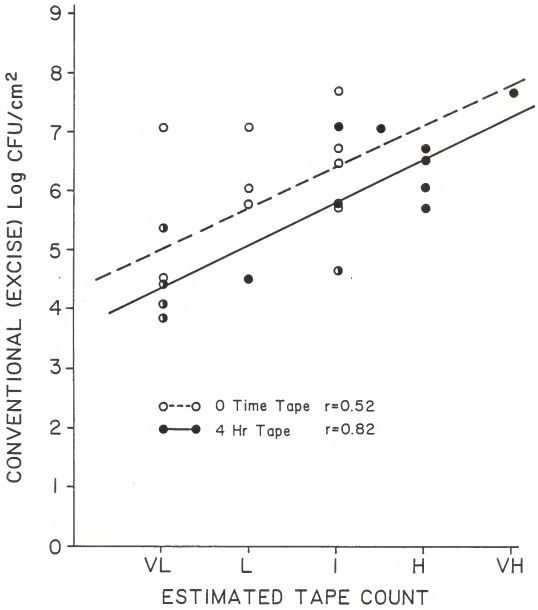

Fig. 8. Surface bacterial count of chicken samples by using tape method vs. excised-blending method.

excised-blending method provided higher counts than swab method when the same sample was used. This may be due to the blending action involved in excised-blending method breaking down bacterial colony into several individual cells (Brown and Baird-Parker, 1982).

The Comparison of 0 Hour Direct Tape Count and 4 Hour Direct Tape Count with Two Conventional Methods on Pork Surfaces

Twenty-two pork samples were used to estimate the microbial loads on surfaces. The results obtained were similar to those from chicken samples. Both swab and excised-blending methods were more correlated with 4 hr direct tape than 0 hr direct tape count. Table 1 shows correlation coefficients between 0 hr direct tape count and swab method was 0.80; whereas the 4 hr direct tape count and swab method was 0.93. Results of mesophilic bacterial counts of 22 pork samples by the adhesive tape method and the conventional swab method are presented in Fig. 9. The data indicate that when 4 hr direct tape count is VH, bacterial counts on pork surfaces by swab method are >log 7 CFU/cm^2; when the direct tape count is H, bacterial counts are between log 6-7 CFU/cm^2; I, bacterial counts are between log 5-6 CFU/cm^2; L, bacterial counts are around log 4 CFU/cm^2 and VL, bacterial counts are low (ca. log 3 CFU/cm^2).

Table 1 shows the correlation coefficient between 0 hr direct tape count and excised-blending method was 0.82; whereas between the 4 hr direct tape count and excised-blending method it was 0.87. The difference of correlation coefficients between these two paired variables was not as large as other comparisons. The data of Fig. 10 indicate that when 4 hr direct tape count is VH, bacterial counts by excised-blending method on pork surfaces are >log 8 CFU/cm^2; when the direct tape count is H, bacterial counts are high (ca. log 7 CFU/cm^2); I, bacterial counts are around log 6 CFU/cm^2; L, bacterial counts are

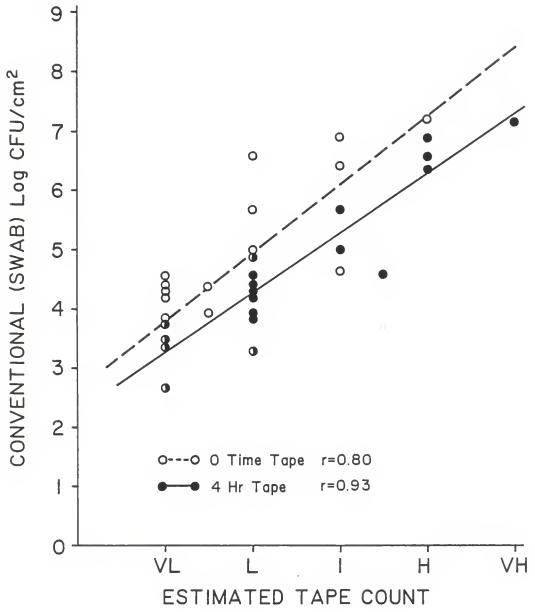

Fig. 9. Surface bacterial count of pork samples by using tape method vs. swab method.

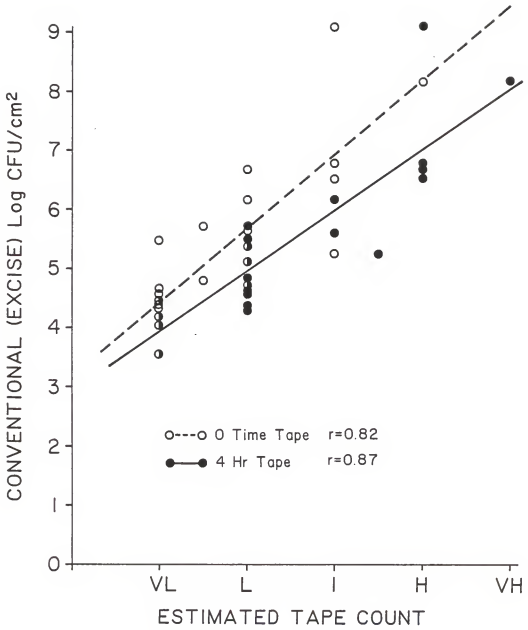

Fig. 10. Surface bacterial count of pork samples by using tape method vs. excised-blending method.

between log 4-5 CFU/cm^2 and VL, bacterial counts are between log 3-4 CFU/cm^2. Excised-blending method gave higher results than swab method for pork samples. This same result was found for chicken. Excise method yielded higher counts than swab method which agreed with the results obtained by Lazarus et al. (1977) and Yokoya and Zulzke (1975) who made the comparisons on beef carcasses.

Odor Profiles Related to Microbial Growth on Chicken Surfaces

In order to relate bacterial counts on chicken surfaces to shelf life of chicken parts, bacterial counts of chicken surfaces were evaluated by the 0 hr direct tape method, 4 hr direct tape method and swab method compared with odor scores.

Microbial counts of 0 day chicken samples by swab method had higher correlation with odor scores than counts obtained by tape method. However, after 3 days storage of chickens at 7°C, odor scores had higher correlation with the microbial estimation using 0 hr direct tape count than using swab method. Table 1 shows that correlation coefficient between odor score and swab method at 0 day and after 3 days cold storage is the same ($r = 0.80$). The 4 hr direct tape count gave lower correlation coefficients in both 0 day ($r = 0.66$) and 3 days storage ($r = 0.67$) chicken samples, but they were still statistically significant ($p < 0.05$).

The relationships of off-odor and microbial contamination on the surface as evaluated by the swab method at 0 day and 3 days storage are shown in Figs. 11 and 12. Odor scores of chicken had a direct correlation with microbial counts. At 0 day, samples with fresh odor had log 3-4 CFU/cm^2, slight off-odor had log 5 CFU/cm^2, moderate off-odor had log 6-7 CFU/cm^2, strong off-odor had log 7-8 CFU/cm^2 and extreme off-odor had log 8-9 CFU/cm^2. The correlation

coefficients of 0 day odor scores and 3 days odor scores with the swab method were both 0.80. The 3 day old samples had higher bacterial counts and more undesirable odor scores as expected.

The relationships between off-odor and microbial contamination on surface evaluated by 0 hr direct tape count as well as 4 hr direct tape count at 0 day and 3 days cold storage are shown in Figs. 13 and 14. Odor scores of chicken also had a direct correlation with microbial counts using the 0 hr direct tape method and the 4 hr direct tape method. At 0 day, microbial loads as evaluated by 0 hr direct tape count were more highly correlated with odor scores ($r = 0.78$) than with 4 hr tape evaluation ($r = 0.66$). By the 0 hr direct tape sampling method, samples with fresh odor had VL-L, slight off-odor had L-I, moderate off-odor had slightly higher than I, strong off-odor had H and extreme off-odor had close to VH. The 4 hr direct tape when correlated with odor scores showed higher counts than 0 hr tape. For 3 day old samples the correlation coefficient with odor score was 0.89 and 0.67, for 0 hr direct tape count and 4 hr direct tape count, respectively. The data presented in Fig. 14 show that using 0 hr direct tape sampling method, samples with fresh odor had VL, slight off-odor had L, moderate off-odor had L-I, strong off-odor had I-H and extreme off-odor had H. Also, the 4 hr direct tape showed higher counts than 0 hr tape. These data indicated that the direct tape count method can predict spoilage potential of chicken.

Odor Profiles Related to Microbial Growth on Pork Surfaces

In order to relate bacterial counts on pork surfaces to shelf life of pork chops, bacterial counts of pork surfaces were evaluated by the 0 hr direct tape method, 4 hr direct tape method and swab method and the relationship with odor scores was determined.

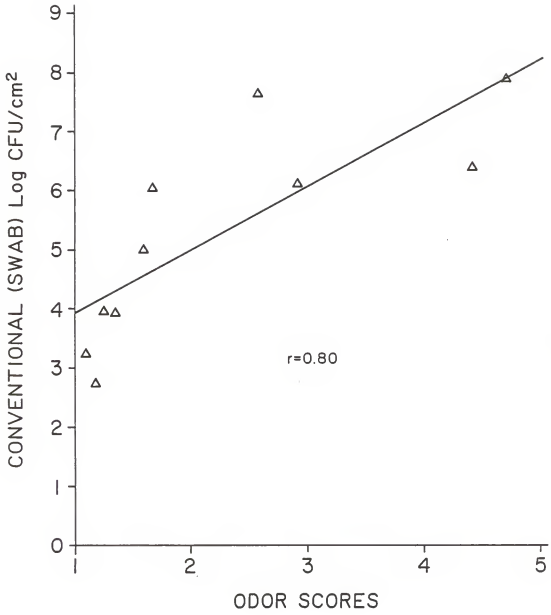

Fig. 11. Odor profiles vs. microbial counts using swab method of chicken samples at zero day.

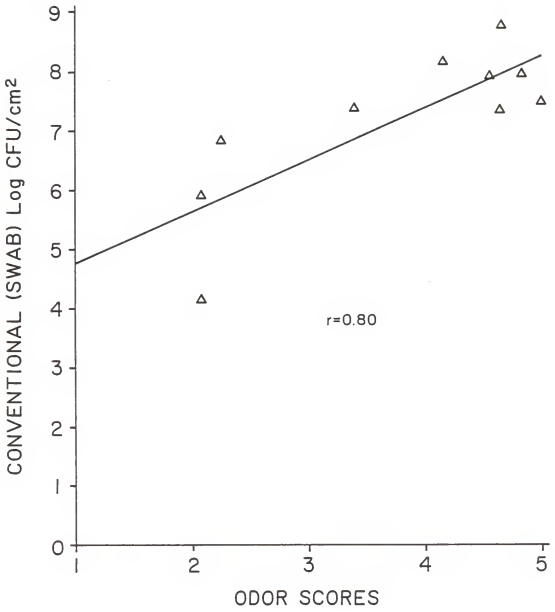

Fig. 12. Odor profiles vs. microbial counts using swab method of chicken samples after 3 days storage at 7°C.

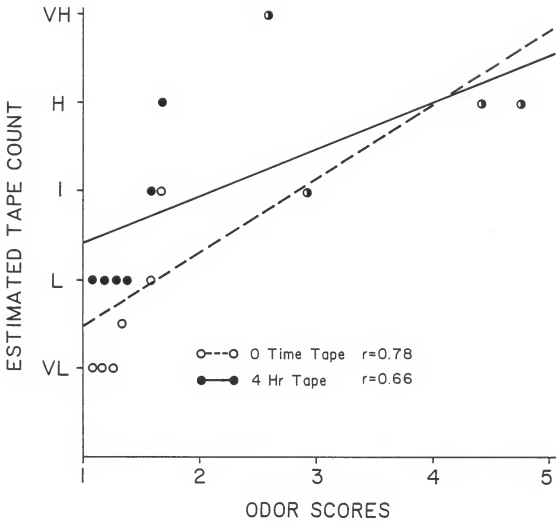

Fig. 13. Odor profiles vs. microbial counts using tape method of chicken samples at zero day.

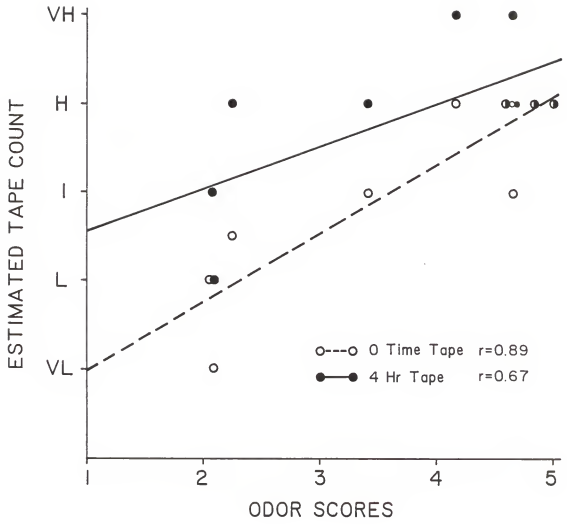

Fig. 14. Odor profiles vs. microbial counts using tape method of chicken samples after 3 days storage at 7°C.

Table 1 shows microbial counts on pork surfaces by swab method had higher correlation with odor scores than those obtained by tape method at both 0 day and 3 days storage of pork samples at 7°C. Correlation coefficients between odor score and swab method at 0 day was 0.91, and after 3 days cold storage was 0.85. The 4 hr direct tape count gave low correlation coefficients in both 0 day ($r = 0.76$) and 3 days storage ($r = 0.07$) pork samples.

The relationships of off-odor and microbial contamination on surface evaluated by swab method at 0 day and 3 days storage are shown in Figs. 15 and 16. Odor scores of pork had a direct correlation with microbial counts. At 0 day, samples with fresh odor had log 4-5 CFU/cm^2, slight off-odor had log 5-6 CFU/cm^2, moderate off-odor had log 6-7 CFU/cm^2, strong off-odor had log 7-8 CFU/cm^2 and extreme off-odor had >log 8 CFU/cm^2. The 3 day old pork samples had higher bacterial counts and more undesirable odor as expected.

The relationships between off-odor and microbial loads on surface by 0 hr direct tape count as well as 4 hr direct tape count at 0 day and 3 days cold storage are shown in Figs. 17 and 18. At 0 day, microbial loads by 0 hr direct tape count were more highly correlated with odor scores ($r = 0.87$) than 4 hr tape ($r = 0.76$). By 0 hr direct tape sampling method, samples with fresh odor had VL, slight off-odor had VL-L, moderate off-odor had L-I, strong off-odor had I-H, and extreme off-odor had H. The 4 hr direct tape showed higher counts than 0 hr tape. For 3 day old samples the correlation coefficients with odor score were 0.40 and 0.07, for 0 hr direct tape count and 4 hr direct tape count, respectively. The data in Fig. 18 show that using 0 hr direct tape sampling method, samples with slight off-odor had L-I, moderate off-odor had slightly higher than I, strong off-odor had I-H, and extreme off-odor had close to H. Also, the 4 hr direct tape showed higher counts than 0 hr tape.

Fig. 18 shows the odor evaluation compared with microbial counts by tape

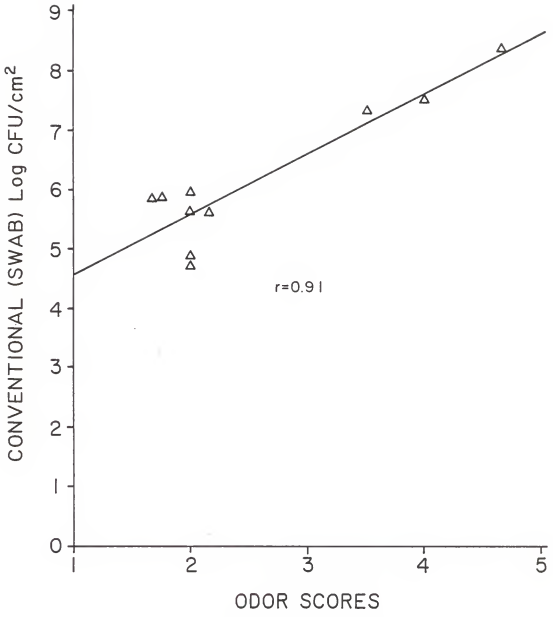

Fig. 15. Odor profiles vs. microbial counts using swab method of pork samples at zero day.

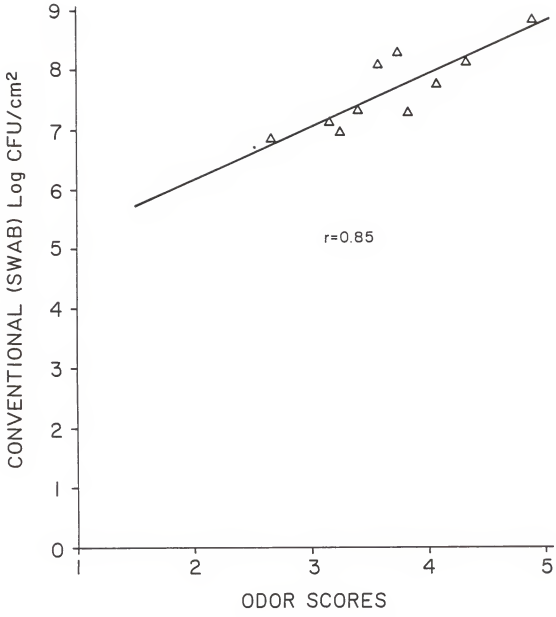

Fig. 16. Odor profiles vs. microbial counts using swab method of pork samples after 3 days storage at 7°C.

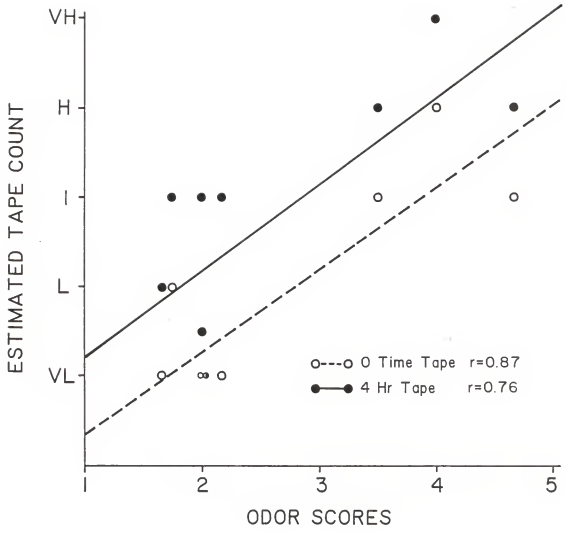

Fig. 17. Odor profiles vs. microbial counts using tape method of pork samples at zero day.

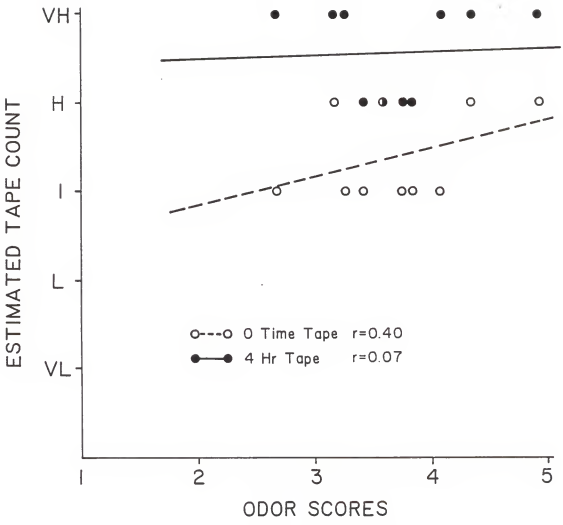

Fig. 18. Odor profiles vs. microbial counts using tape method of pork samples after 3 days storage at 7°C.

method of 10 pork samples after 3 days storage at 7°C. However, the linear regression lines of both odor scores vs. 0 hr direct tape count and odor scores vs. 4 hr direct tape count are very flat. The correlation coefficients between both comparisons were low and also statistically insignificant ($p > 0.05$). This may be due to all 10 pork samples after 3 days storage having high bacterial contamination on the surfaces (both 0 hr direct tape count and 4 hr direct tape count showed intermediate count or higher), and the odor scores averaged from 6 panelists varied from moderate off-odor to extreme off-odor. For pork samples after 3 days storage, the level of surface contamination estimated by tape method is not able to significantly differentiate different degrees of pronounced off-odor, if the microbial counts are high.

The Prediction of Development of Off-Odor by 0 Day Microbial Counts

To predict off-odor development after cold storage, surface microbial counts by the 0 hr direct tape count, 4 hr direct tape count and the swab method at 0 day were correlated with odor score of the same sample. Tables 2 and 3 show correlation coefficients between surface microbial counts at 0 day and the odor evaluation of 0 day and 3 days storage for chicken and pork samples. Surface microbial counts by the three methods at 0 day were more highly correlated with odor scores of 3 days storage chicken samples than those of 0 day chicken samples. For pork samples, correlation coefficients of 0 day microbial counts by all the three methods and odor scores of storage for 3 days were lower than those of 0 day microbial counts and odor scores of 0 day. However, significantly positive correlations ($p < 0.05$) were found between 0 day surface microbial counts and odor evaluation of both 0 day and 3 days storage pork samples. The direct tape count methods and the swab method can be used to predict odor of chicken and pork samples, and the odor prediction for chicken

Table 2. Correlation coefficients between surface microbial counts at 0 day and the odor evaluation of 0 day and 3-day storage chicken samples.

	Odor evaluation	
	0 day	3-day
0h tape	0.78	0.84
4h tape	0.66	0.85
Swab	0.80	0.83

Table 3. Correlation coefficients between surface microbial counts at 0 day and the odor evaluation of 0 day and 3-day storage pork samples.

	Odor evaluation	
	0 day	3-day
0h tape	0.87	0.68
4h tape	0.76	0.58
Swab	0.91	0.72

was better than that of pork.

Shelf Life of Retail Chicken and Pork Meats

When comparing the odor scores and microbial contamination on surfaces with samples collected at different days and after 3 days of storage for chicken and pork samples, the following suggestions were made. The sell-by date stamped on the package of meat is a good guideline for consumers to use. Meat should be consumed before that date when odor is still fresh and microbial counts are low (log 2-4 CFU/cm^2). However, the development of off-odors (increased odor scores) and high surface microbial contamination (log 6-8 CFU/cm^2) lowered meat quality and accelerated spoilage potential, when the meat was over the sell-by date. When meat samples were within the sell-by dates, the longer storage at refrigerated temperature (7°C), the higher the off-odor scores and the higher number of total bacterial counts from surfaces. For pork samples evaluated on the sell-by date or over the date by 1 day, slight off-odors were detected and microbial loads on surfaces were intermediate. The meat quality may be acceptable but the meat should be consumed as soon as possible. There were some chicken samples with low off-odor score but heavy microbial contamination. This may be due to only a small portion of bacterial strains contributing to off-odors. Microbial behavior during storage is affected not only by the size of the initial contamination but also by the nature of the organisms involved. Cox et al. (1975) suggested that the intensity of odor development during spoilage of poultry meat by Pseudomonas was more closely associated with the nature of the metabolism of the particular organism, than with differences in rates of multiplication among species or with various components of the poultry carcass.

The Value of The Adhesive Tape Method

The adhesive tape method tested in this study was designed for estimating microbial loads on surfaces and is especially valuable in meat industry. The tape method is simple, non-destructive, and able to provide a good assessment of the potential shelf life of the meat. No dilution and plating procedures are needed. The tape method is easy to perform, requires little time and material, and saves working and incubation space. Tape can be used to evaluate microbial loads at different incubation temperatures and on different types of agar media, e.g., selective or differential medium for a specific bacterial count. It is applicable to red meat (beef and pork), chicken and cured meat surfaces. The 24 hr tape-counting method can quantitatively measure surface bacterial population, and 8 hr of incubation for tape counted by the laboratory counter is enough for examining microbial quality of meat without aid of microscopy. A 4 hr direct tape count correlated better with both conventional methods than 0 hr direct tape count of chicken and pork surface samples. The 0 hr and 4 hr direct tape count methods can also be applied to predict the intensity of odor development for chicken and pork samples. The increasing number of bacteria from 0 hr to 4 hr with the tape staining technique shows the viability of the bacterial cells. A gram-staining procedure can be effectively employed to distinguish microbes from other non-microbial surface residues. The proportion of gram-positive and gram-negative cells of surface microorganisms can also be estimated.

CONCLUSIONS

Based on the results of this study, the following conclusions are made:

1. The tape method is a rapid and non-destructive surface sampling method that can monitor the bacteriological quality of poultry and red meats. It is easy to perform, flexible to different meat surfaces, and saves materials and spaces.

2. The 24 hr tape counting procedure is a quantitative method which can be applied to raw meat as well as cured meat products. Good correlations ($p < 0.01$) were obtained between results of the tape method and two conventional methods (swab and excised-blending methods). When the tape count reached saturation point, bacterial contamination was >log 6 CFU/cm^2 which was an indication of poor quality and spoilage of meat.

3. A 4 hr direct tape count, using staining the adhesive tape before it was examined microscopically, had significantly positive correlations ($p < 0.01$) with both conventional methods (swab and excised-blending) in chicken and pork samples. When the 4 hr direct tape count showed high or very high counts in the determined scale, the bacterial counts were log 6-8 CFU/cm^2. Using this method the surface microbiological quality of meat can be estimated in as fast as 4 hr. Its speed and convenience make it valuable in the practical application for estimating the shelf life of meat products, so those with shorter potential shelf life can be sold first.

4. Odor evaluation had higher correlation with the level of microbial contamination as determined on the surface by swab method than by direct tape count method in chicken and pork samples. When meat samples had fresh odor, bacterial counts on surfaces were log 3-5 CFU/cm^2 (very low to low counts); when meat samples showed strong or extreme off-odors, bacterial counts on

surfaces were log 7-9 CFU/cm^2 (high to very high counts).

5. Odor scores also had a positive correlation with surface microbial counts as determined by the 0 hr ($p < 0.01$) and 4 hr ($p < 0.05$) direct tape count in all meat samples, except pork samples after 3 days storage at 7°C. The direct tape count procedure is useful in predicting spoilage potential of chicken and pork.

6. During cold storage, the potential of using the 0 hr and 4 hr direct tape count as well as the swab method in predicting odor development was better for chicken than for pork.

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APPENDIX

Table A. Linear regression of the tape methods and the conventional methods

Figure no.	Slope	Y intercept
1	5.9688	-6.8219
2	4.5201	-4.1019
3	1.4413	2.2561
4	1.5120	2.7219
7 (0h tape)	1.1777	3.0280
(4h tape)	0.9409	2.6447
8 (0h tape)	0.7023	4.3513
(4h tape)	0.7304	3.6449
9 (0h tape)	1.1539	2.6315
(4h tape)	1.0114	2.2459
10 (0h tape)	1.2628	3.1422
(4h tape)	1.0247	2.9258
11	1.0724	2.8627
12	0.8667	3.9156
13 (0h tape)	0.8390	0.6413
(4h tape)	0.5334	1.8866
14 (0h tape)	0.7734	0.2304
(4h tape)	0.4823	2.0793
15	1.0075	3.5849
16	0.8749	4.4294
17 (0h tape)	0.9240	-0.5803
(4h tape)	0.9687	0.2547
18 (0h tape)	0.3255	2.1955
(4h tape)	0.0597	4.3792

Table B. Odor evaluation of 10 chicken samples at 0 day.

Sample no.	Odor score (mean ± standard deviation)
1	1.08 ± 0.19
2	1.67 ± 0.37
3	4.75 ± 0.25
4	1.58 ± 0.34
5	1.17 ± 0.24
6	1.33 ± 0.24
7	2.92 ± 0.93
8	1.25 ± 0.25
9	2.58 ± 0.67
10	4.42 ± 0.45

Table C. Odor evaluation of 10 chicken samples after 3 days storage at 7°C

Sample no.	Odor score (mean ± standard deviation)
1	2.08 ± 0.45
2	4.58 ± 0.45
3	5.00 ± 0.00
4	4.17 ± 0.37
5	3.42 ± 1.20
6	2.08 ± 0.73
7	4.67 ± 0.37
8	2.25 ± 0.63
9	4.67 ± 0.47
10	4.83 ± 0.24

Table D. Odor evaluation of 10 pork samples at 0 day

Sample no.	Odor score (mean ± standard deviation)
1	2.00 ± 0.41
2	2.00 ± 0.65
3	2.17 ± 0.62
4	1.75 ± 0.48
5	3.50 ± 0.29
6	4.67 ± 0.37
7	4.00 ± 0.50
8	1.67 ± 0.69
9	2.00 ± 0.76
10	2.00 ± 0.82

Table E. Odor evaluation of 10 pork samples after 3 days storage at 7°C

Sample no.	Odor score (mean ± standard deviation)
1	3.42 ± 0.34
2	2.67 ± 0.99
3	3.25 ± 1.11
4	4.08 ± 0.67
5	3.58 ± 0.67
6	4.92 ± 0.19
7	4.33 ± 0.24
8	3.75 ± 1.03
9	3.83 ± 0.37
10	3.17 ± 0.85

APPLICATION OF THE ADHESIVE TAPE METHOD FOR MICROBIAL SAMPLING ON VARIOUS MEAT SURFACES

by

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B.S., Tunghai University, Taiwan, R.O.C., 1979

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ABSTRACT

Mylar adhesive tapes were used to estimate the microbiological quality of meat surfaces. The 24 hr tape-counting procedure was tested on surfaces of chicken wings and cured meat products and then compared with the conventional methods of swab and excised-blending, respectively. Reduction of incubation time from 24 hr to 8 hr was studied with the tape-counting method on chicken and pork samples to estimate microbial loads earlier. Furthermore, a technique of staining tape was developed and microbial counts were made by microscopy to estimate the surface contamination level on chicken and pork samples at 0 hr and 4 hr. The development of off-odor was determined by odor evaluation panels and correlated with microbial levels on surfaces that were examined by 0 hr, 4 hr direct tape count , and conventional swab method to predict the spoilage potential of retail raw meat.

The 24 hr tape-counting procedure had good correlations ($p < 0.01$) with two conventional methods (swab and excised-blending methods) for mesophilic and gram-negative bacterial counts on raw chicken wing samples and cured meat products. The reduction of incubation time to 8 hr for tape-counting procedure provided similar results as use of 24 hr incubation time in estimating surface microbial loads of chicken and pork samples. The 4 hr direct tape count was found to be significant in correlation ($p < 0.01$) with the two conventional methods, and higher correlation coefficients were obtained with swab method. Odor evaluation had a direct positive correlation ($p < 0.05$) with surface microbial counts by swab method, and 0 hr and 4 hr direct tape count methods, except correlations ($p > 0.05$) between odor scores and direct tape counts of pork samples after 3 days storage at 7°C. The significance of using swab method, and

0 hr and 4 hr direct tape count methods to predict odor development for chicken samples ($p < 0.01$) was better than for pork samples ($p < 0.05$).